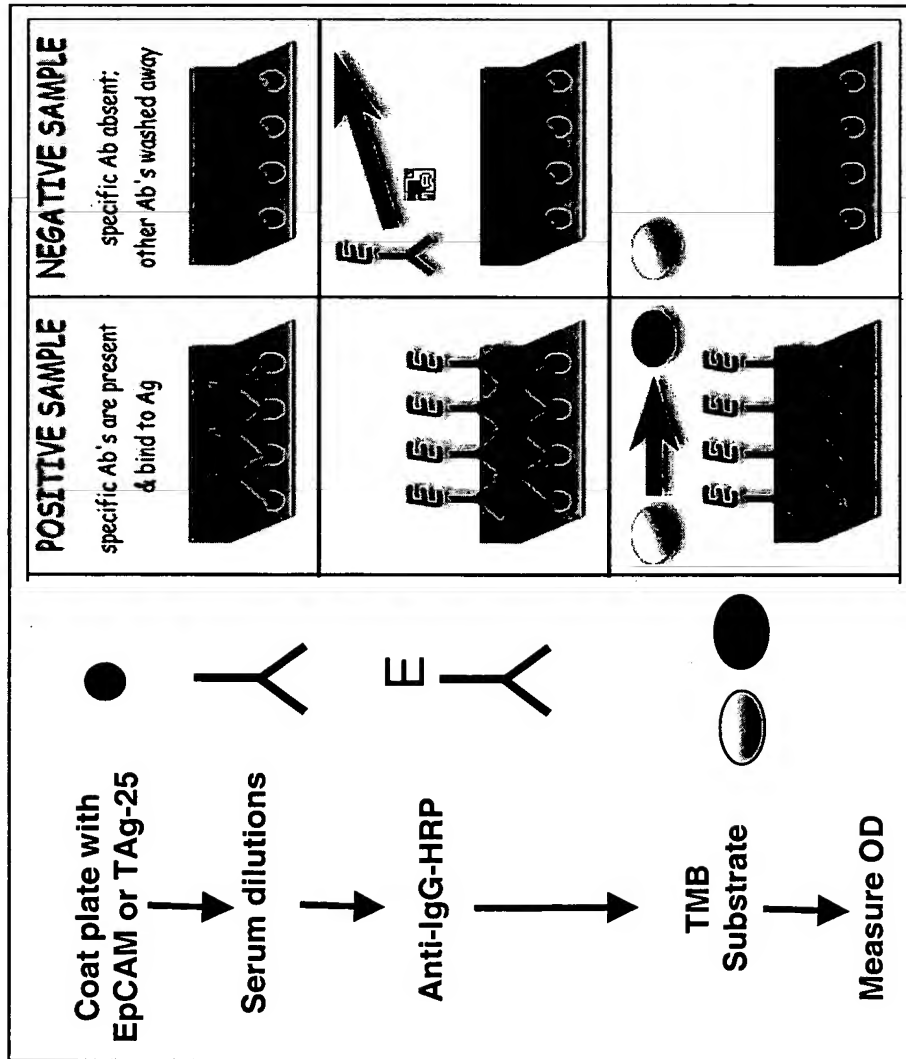
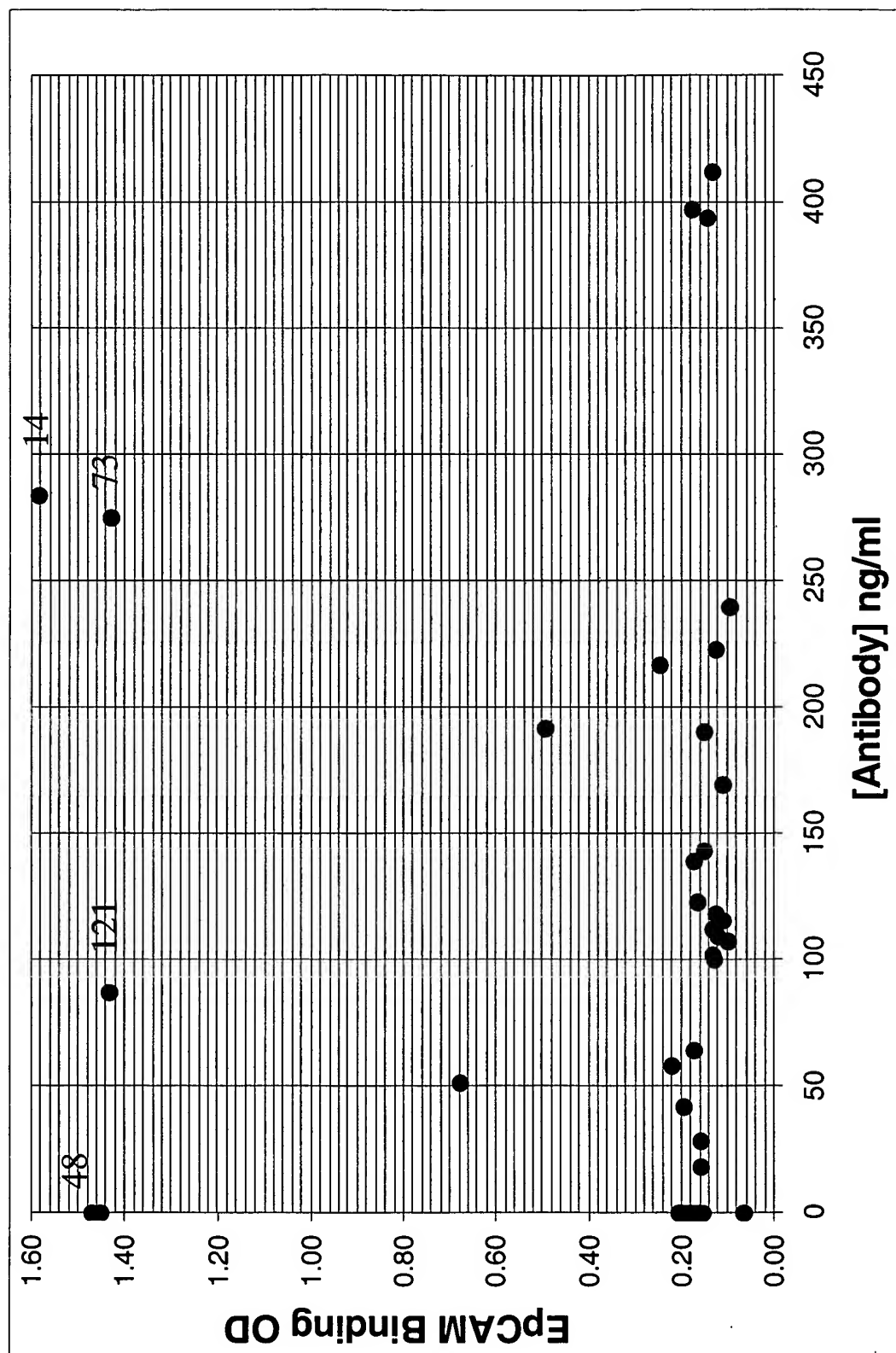


Figure 1



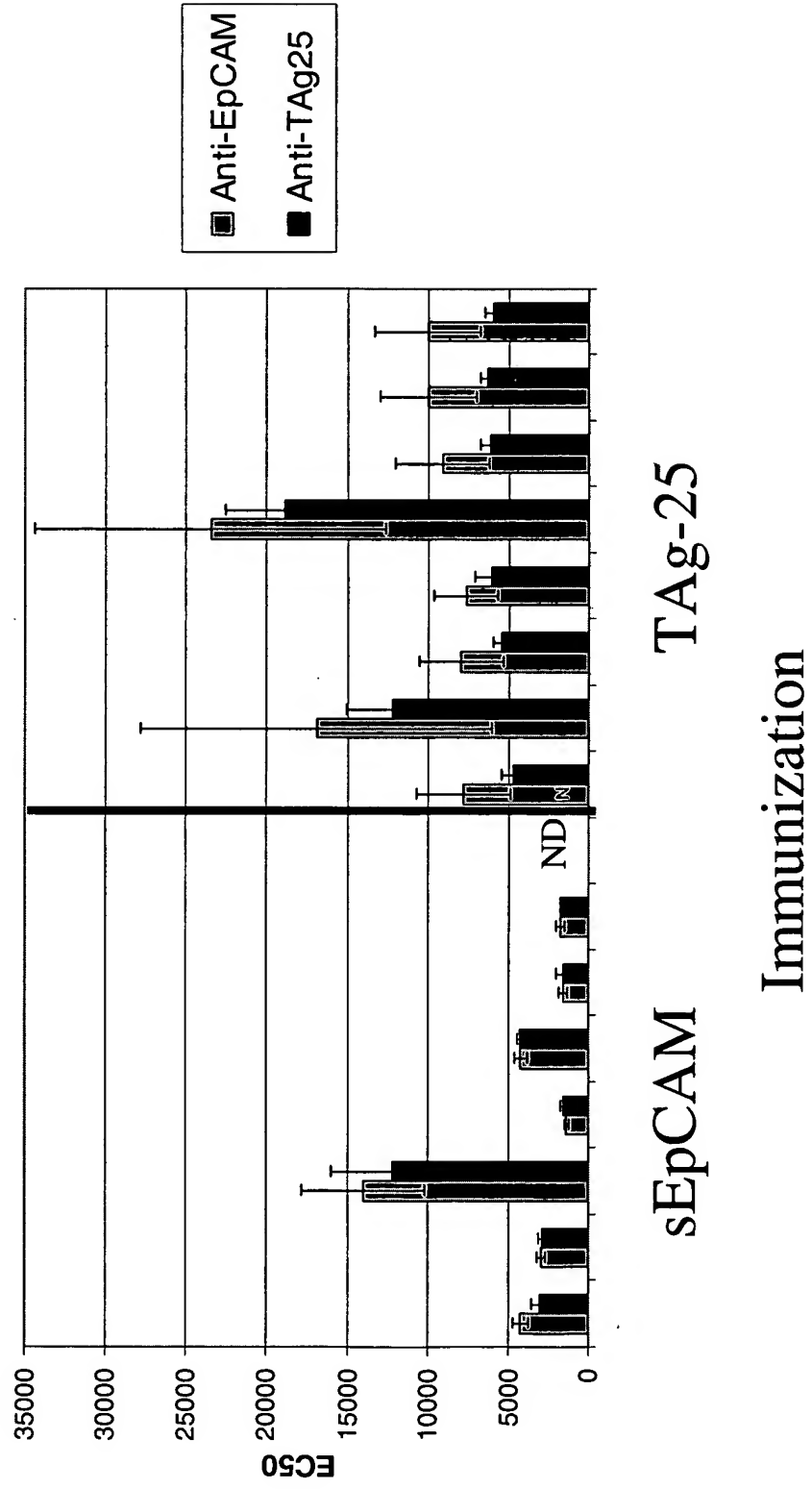
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Figure 2



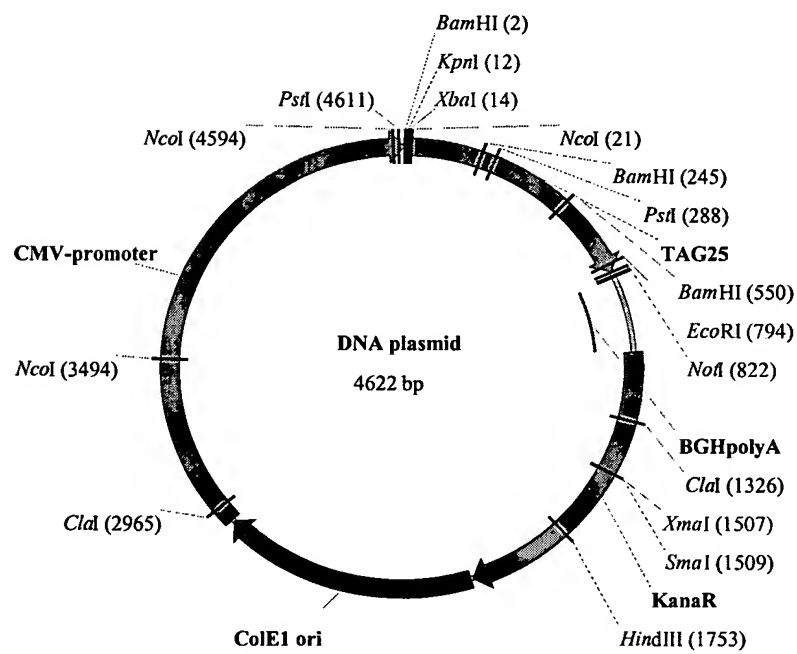
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Figure 3



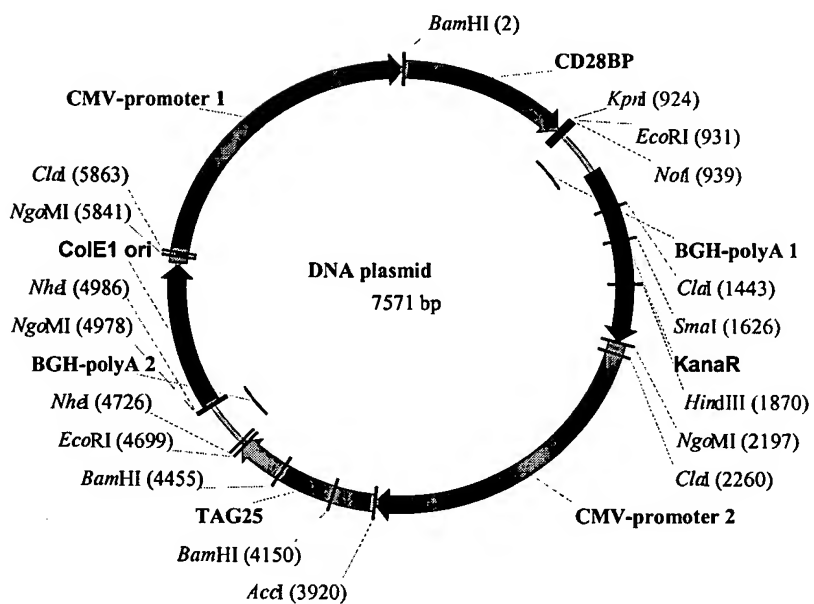
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Figure 4



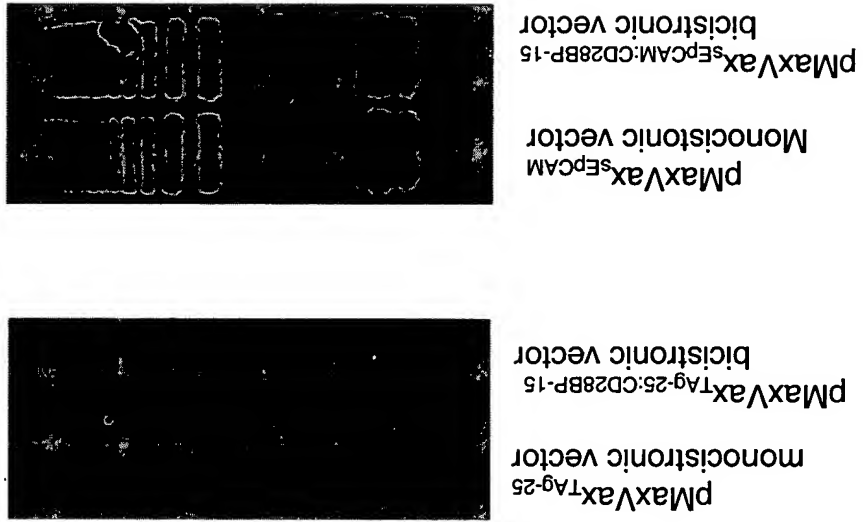
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Figure 5



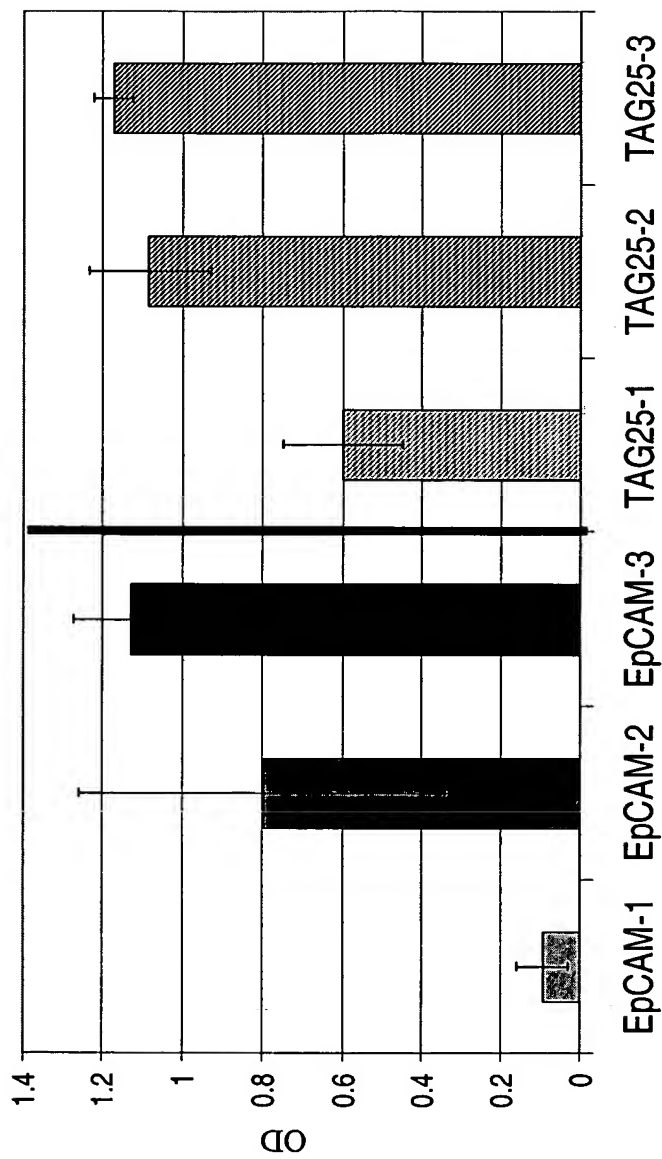
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Figure 6



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Figure 7



Immunization

Figure 8

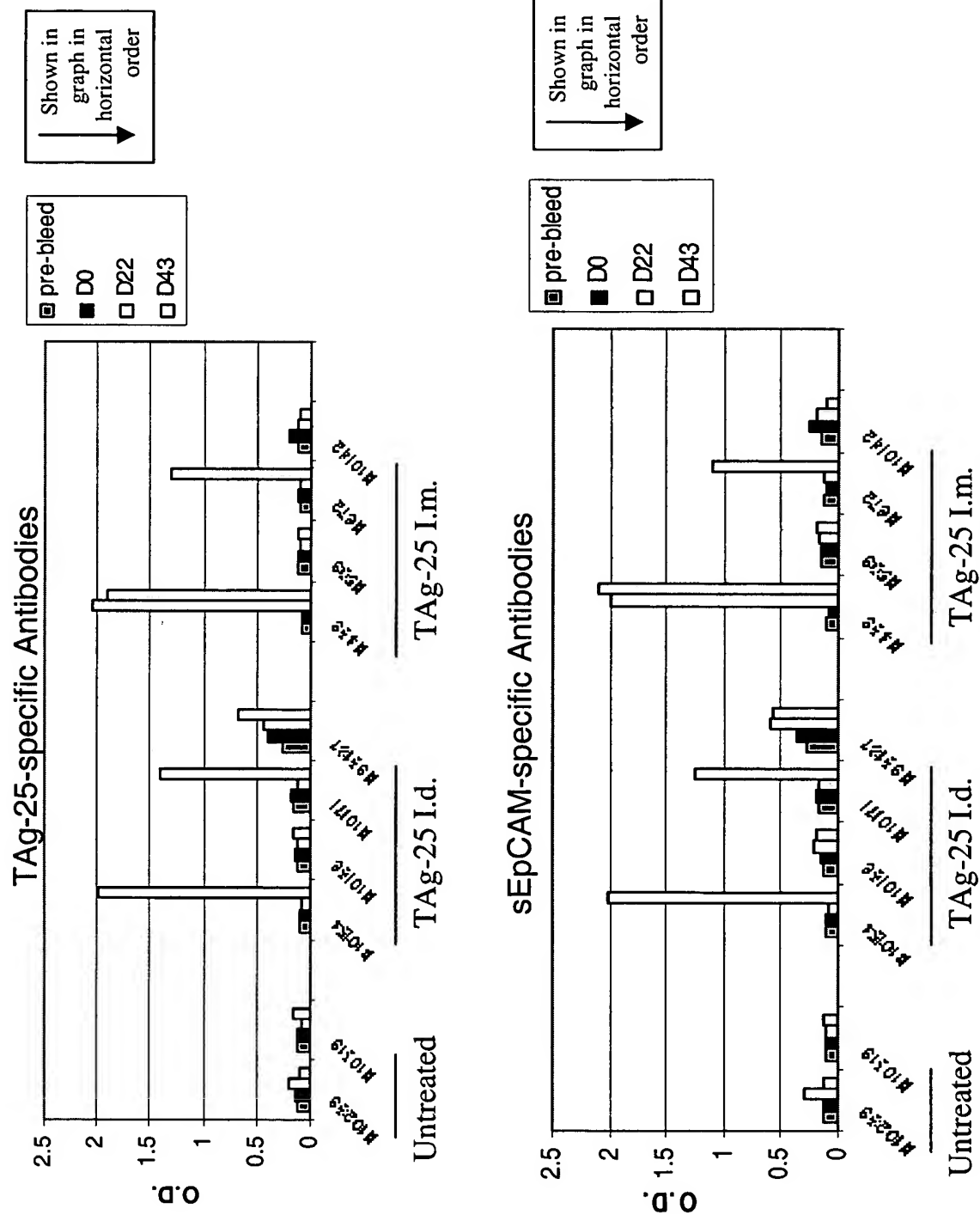




Figure 9A

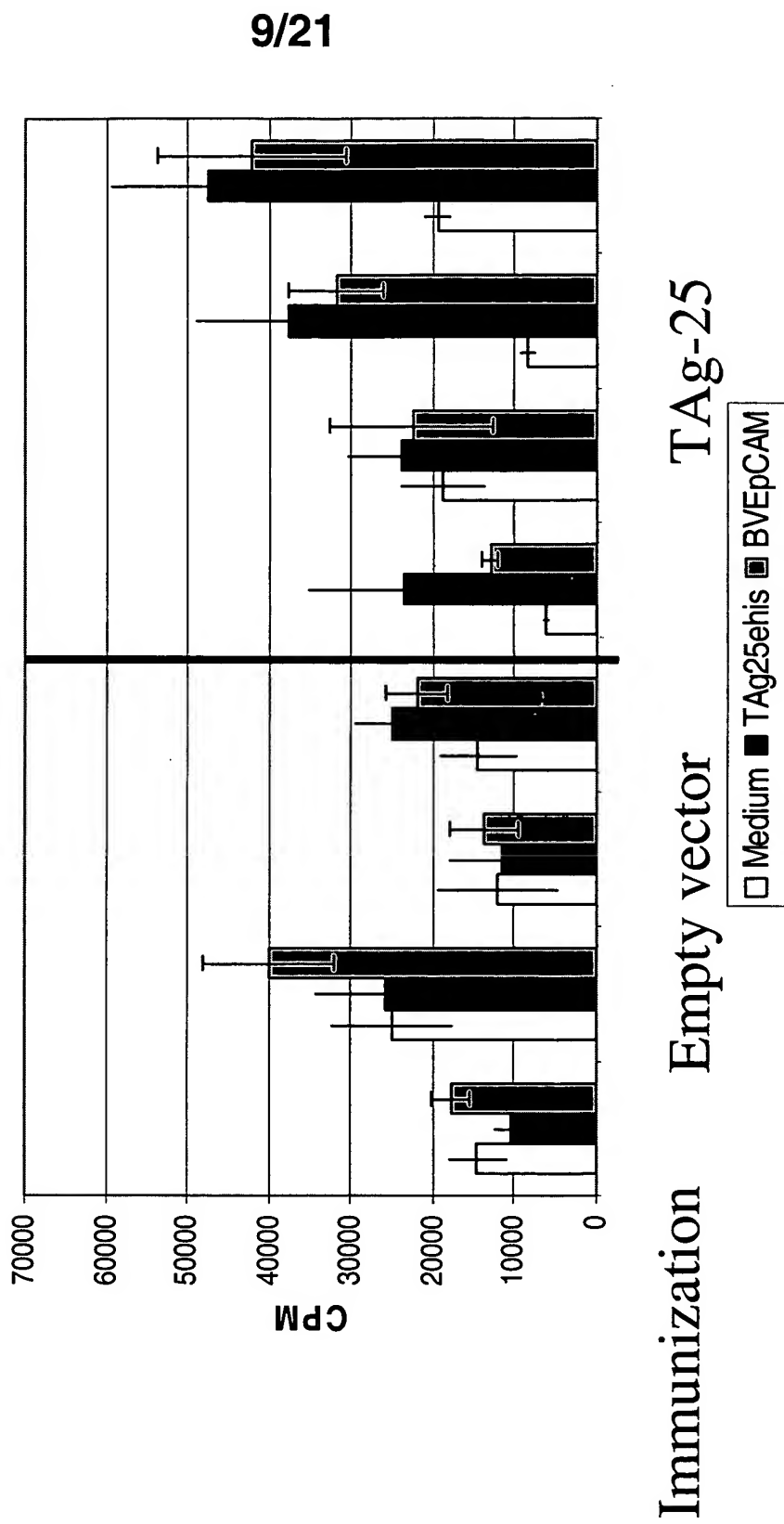
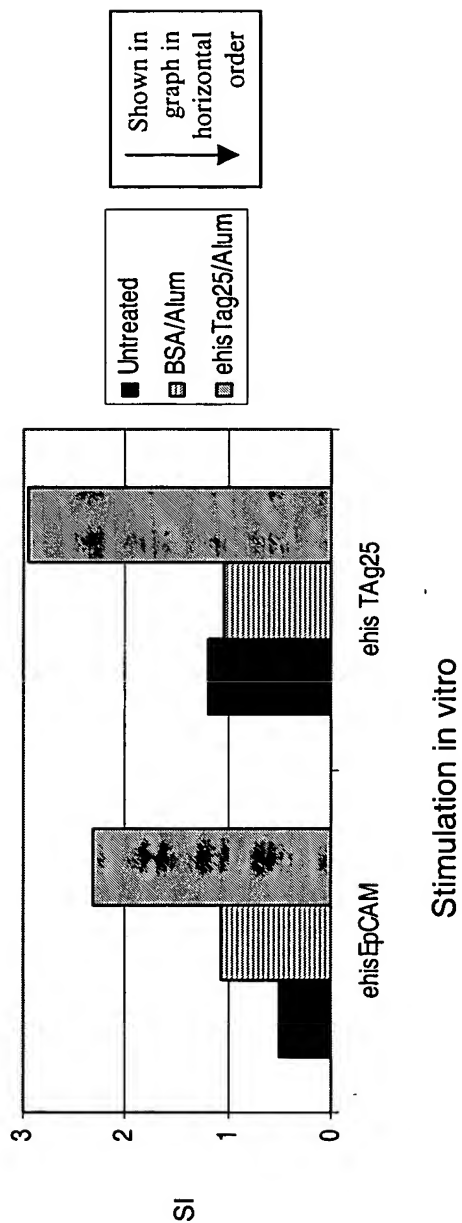


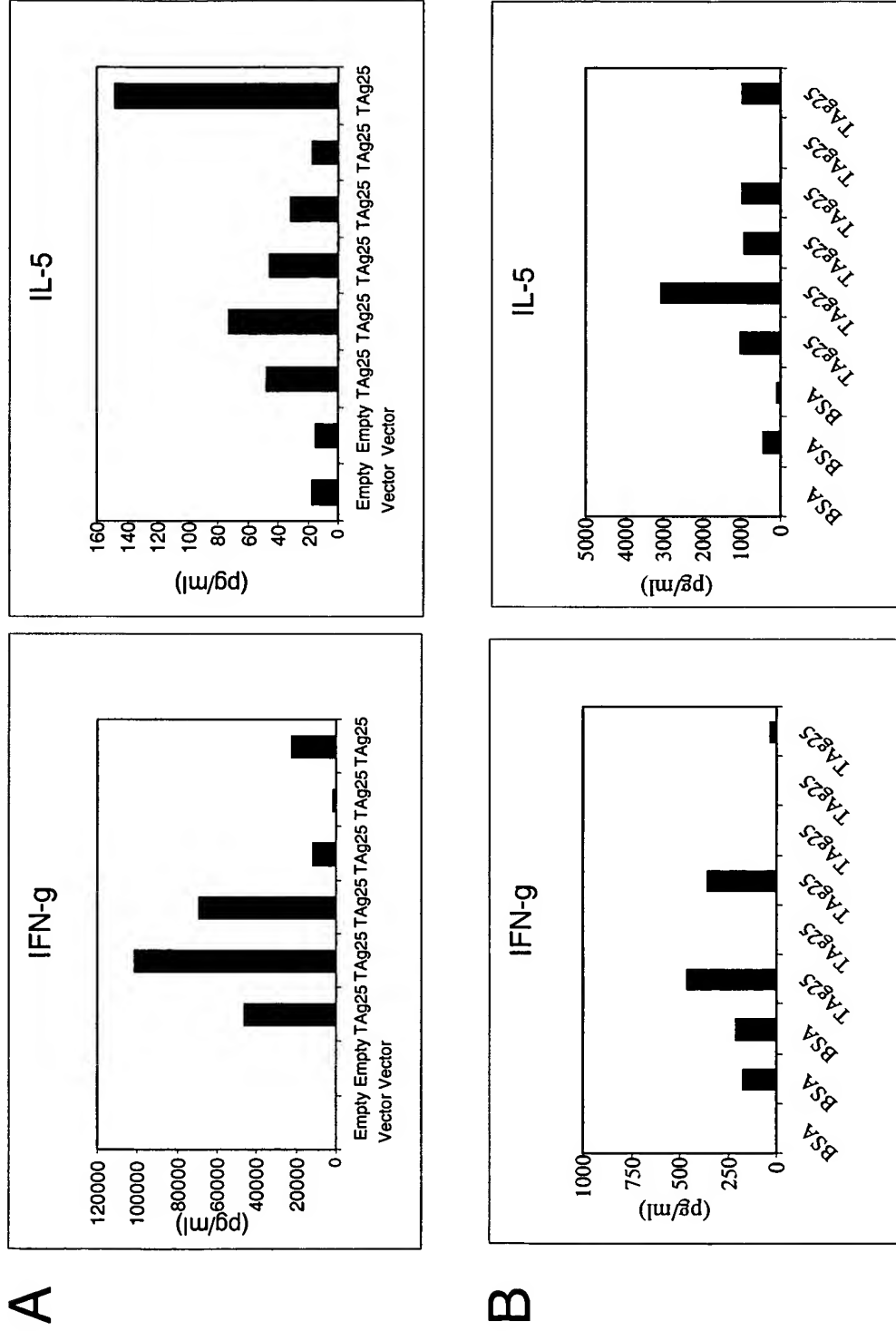
Figure 9B

Protein Vaccine:



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Figures 10A and 10B



Immunization

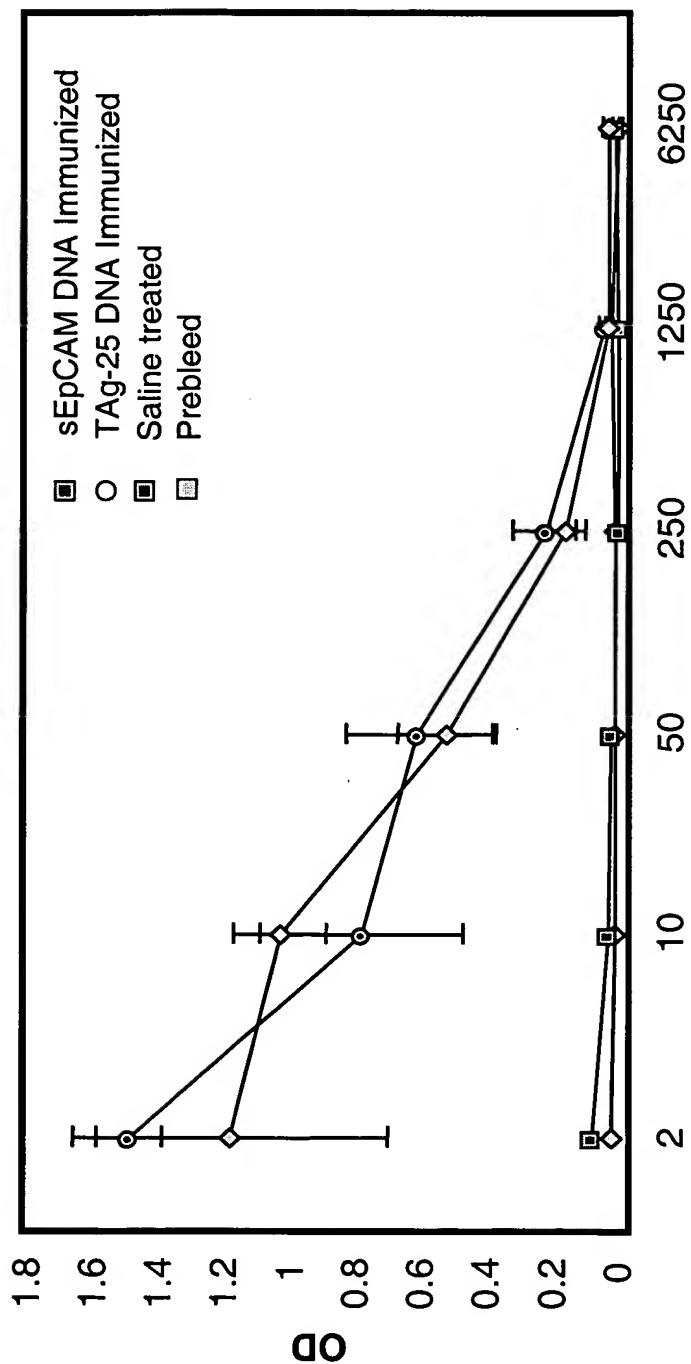
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Figure 11

DNA Treatment	Route	Pre-bleed		1st DNA		2nd DNA		3rd DNA		4th DNA	
		Responders to EpCAM	Responders to TAG25	Responders to EpCAM	Responders to TAG25	Responders to EpCAM	Responders to TAG25	Responders to EpCAM	Responders to TAG25	Responders to EpCAM	Responders to TAG25
Saline	im	0	0	0	0	0	0	0	0	0	0
EpCAM	id	0	0	0	0	1	1	3	3	3	3
EpCAM	im	0	0	1	0	2	2	2	2	2	2
TAG25	id	0	0	0	0	0	0	3	3	3	3
TAG25	im	0	0	0	1	1	1	4	4	4	4

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Figure 12



Reciprocal Serum Dilution after 4<sup>th</sup> immunization with DNA vector (1 mg/per dose)

Figure 13

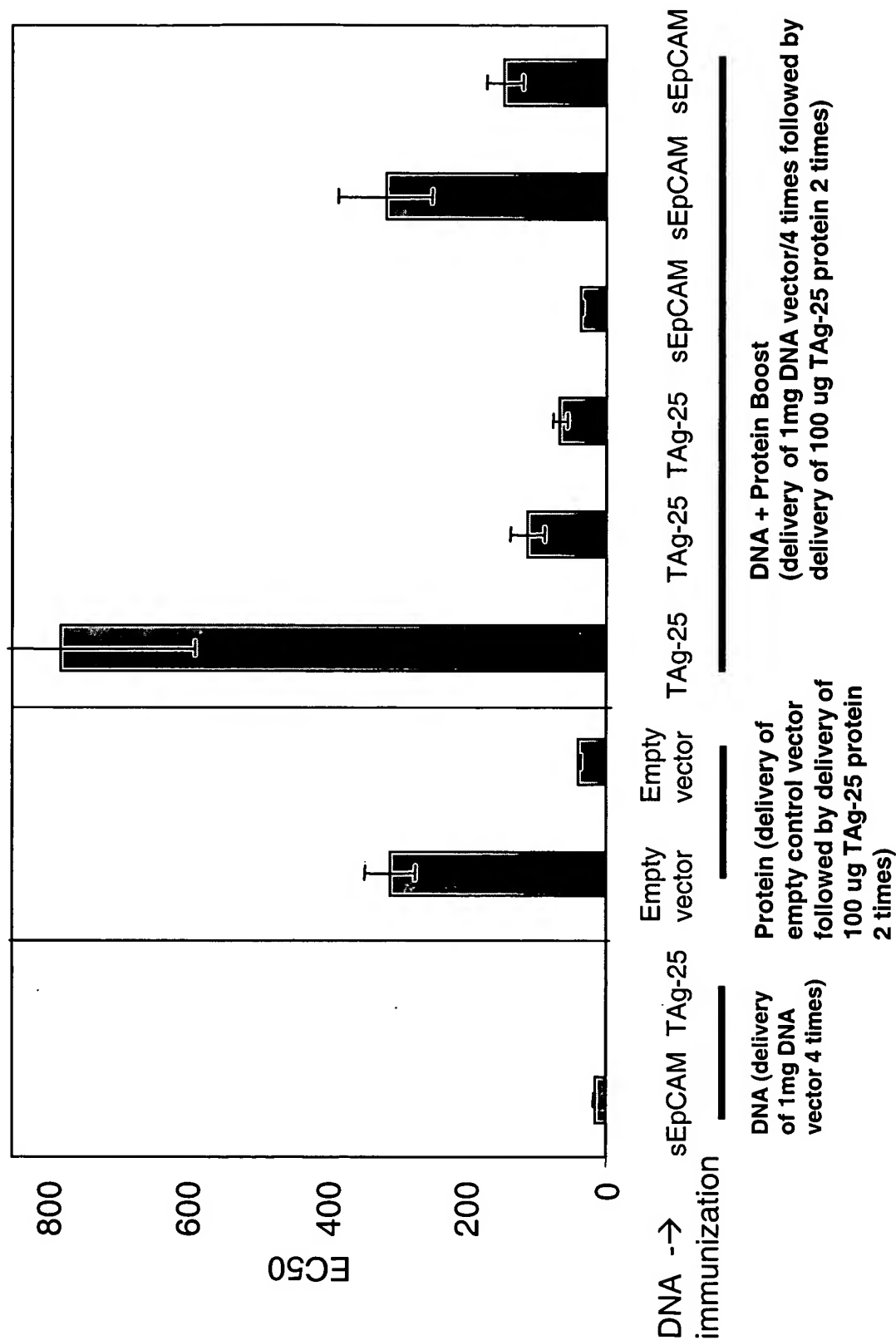
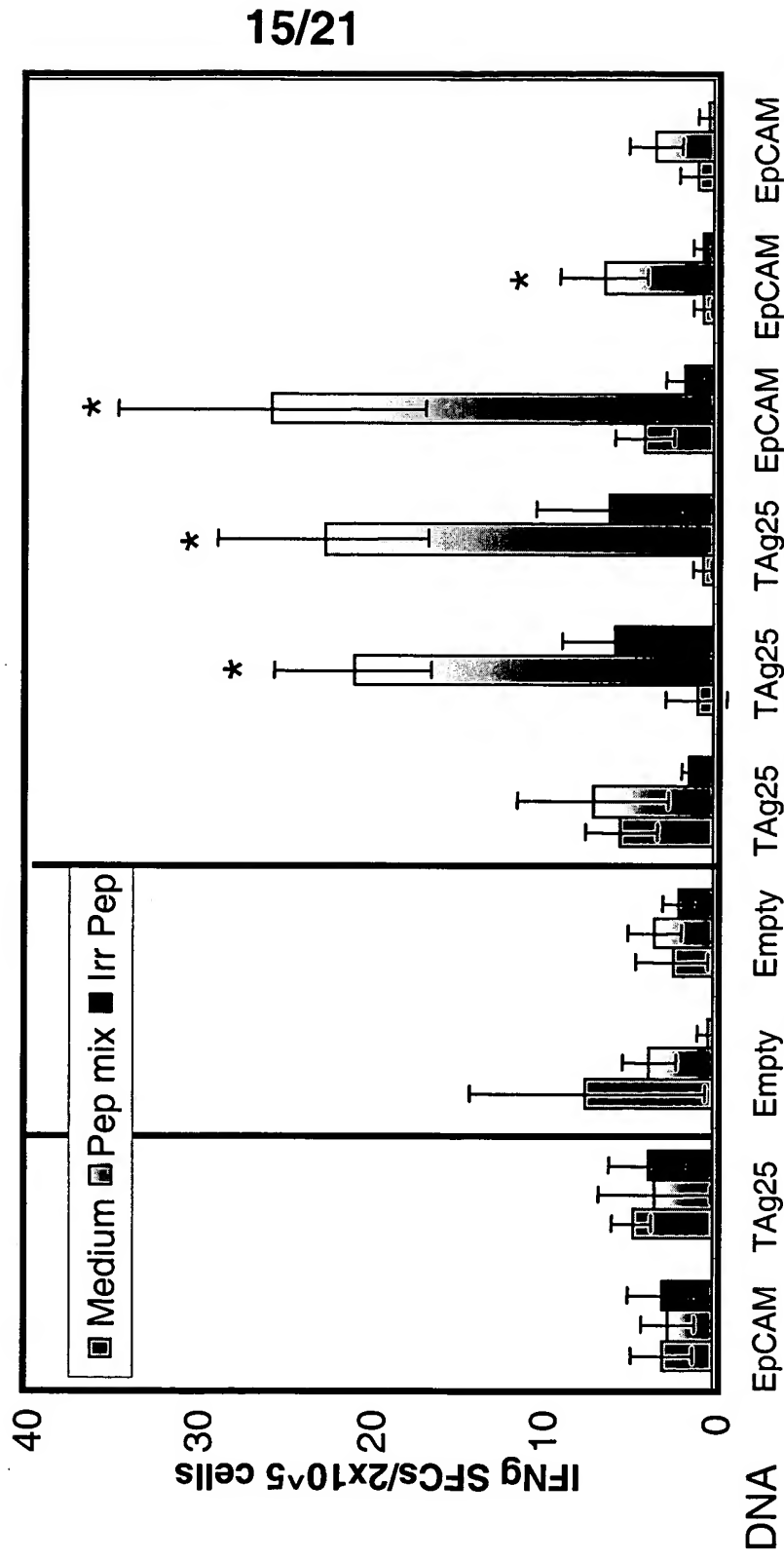


Figure 14



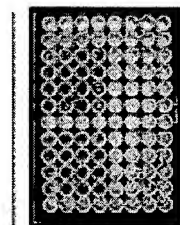
**DNA      Protein      DNA + Protein Boost**

After Last Protein Boost      \* Significantly higher than negative ctrl

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Figure 15

- Preactivate PBMCs +/- antigen in tubes (  $2 \times 10^6$ /ml) for 16-24hr
- Coat PVDF membrane with capture anti-IFN $\gamma$  Ab

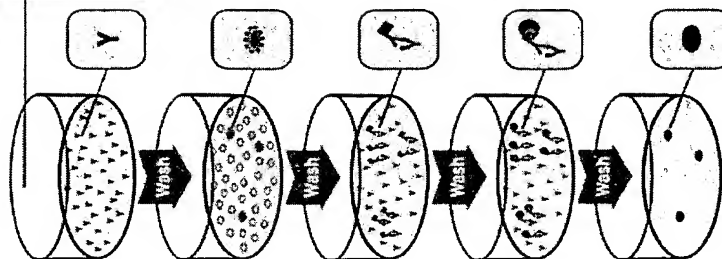


- Block
- Add cells in quadruplicates

- Biotinylated capture Ab

- Streptavidin HRP

- Substrate AEC



Readout: IFN $\gamma$  Spot Forming Cells

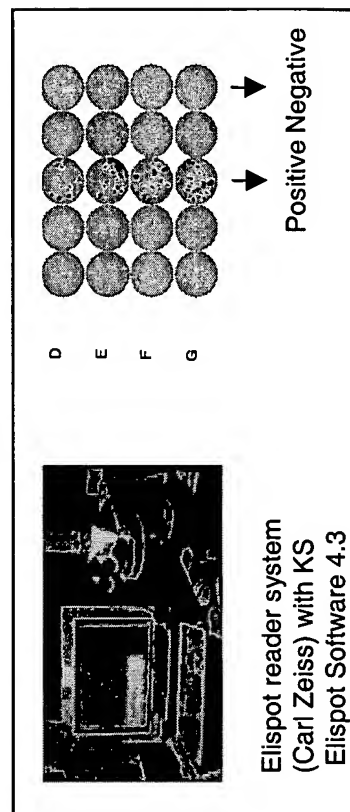
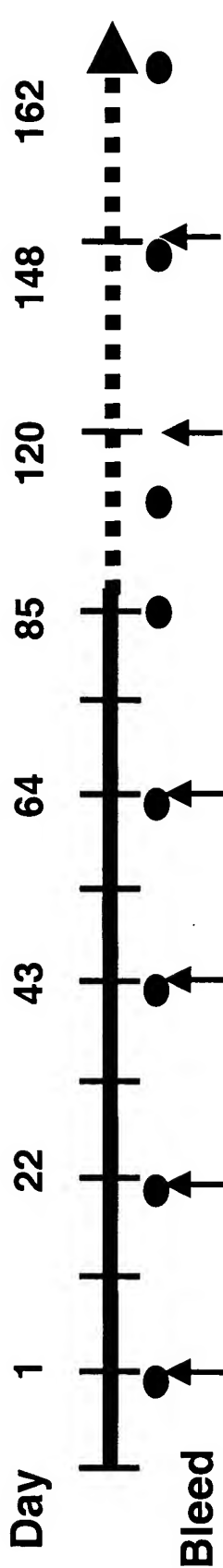




Figure 16



**2mg DNA 4x every 3 wks**

DNA immunizations (n=5):

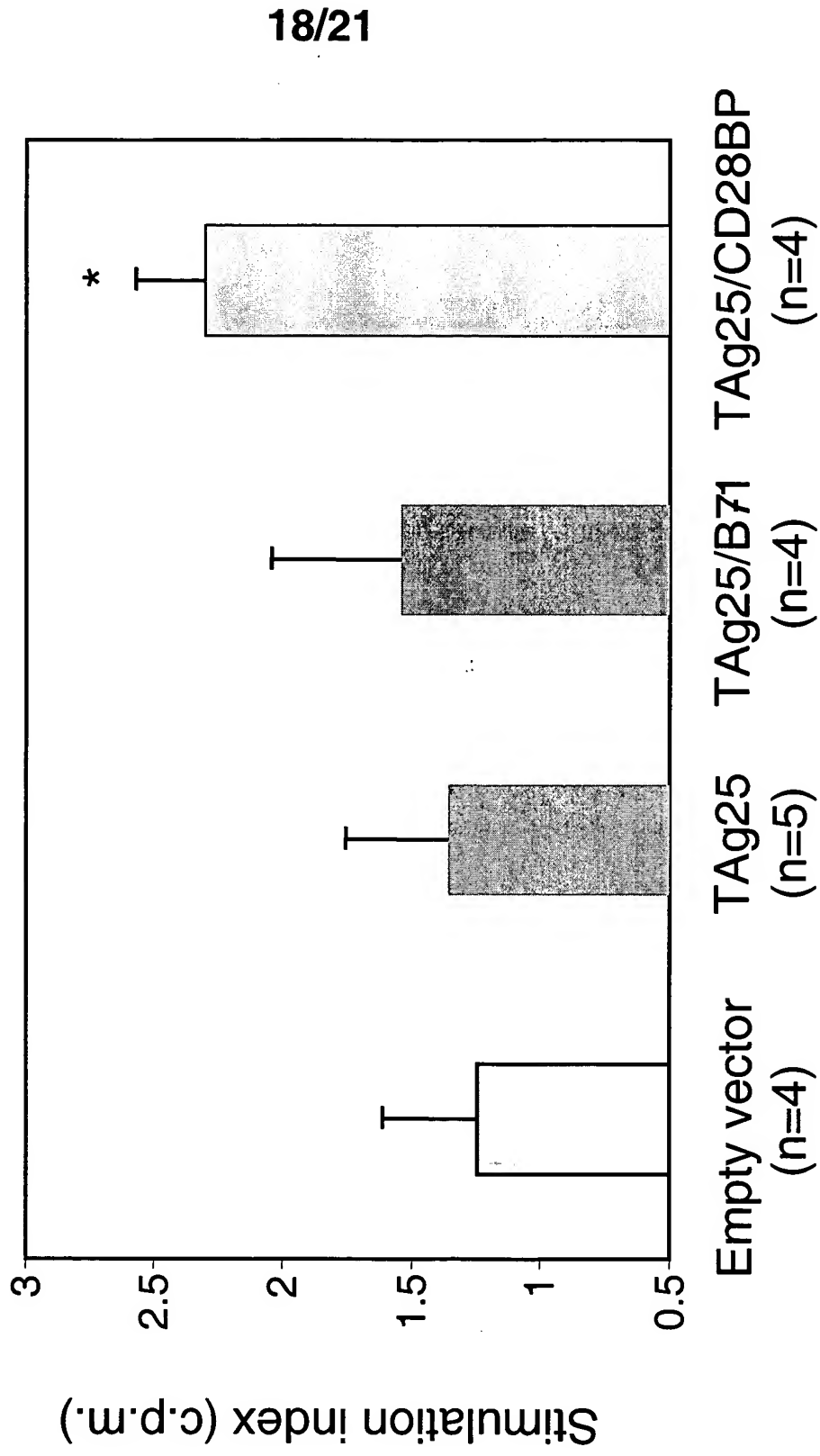
- Empty vector i.m. or i.d.
- TAg25 i.m. or i.d.
- TAg25/hB7.1 i.m. or i.d.
- TAg25/CD28BP i.m. or i.d.
- TAg25 + control DNA i.m.
- TAg25 + hB7.1 i.m.
- TAg25 + CD28BP i.m.

**100ug TAg25 protein in 2mg Alum**  
**2x every 4wks; i.m.**  
 Given to 20 monkeys previously  
 treated with DNA i.d.



Animal Species:  
 Macaca fascicularis

Figure 17



Immunization i.d.

Restimulation using a mixture of 9-11  $\alpha\alpha$  EpCAM-specific peptides

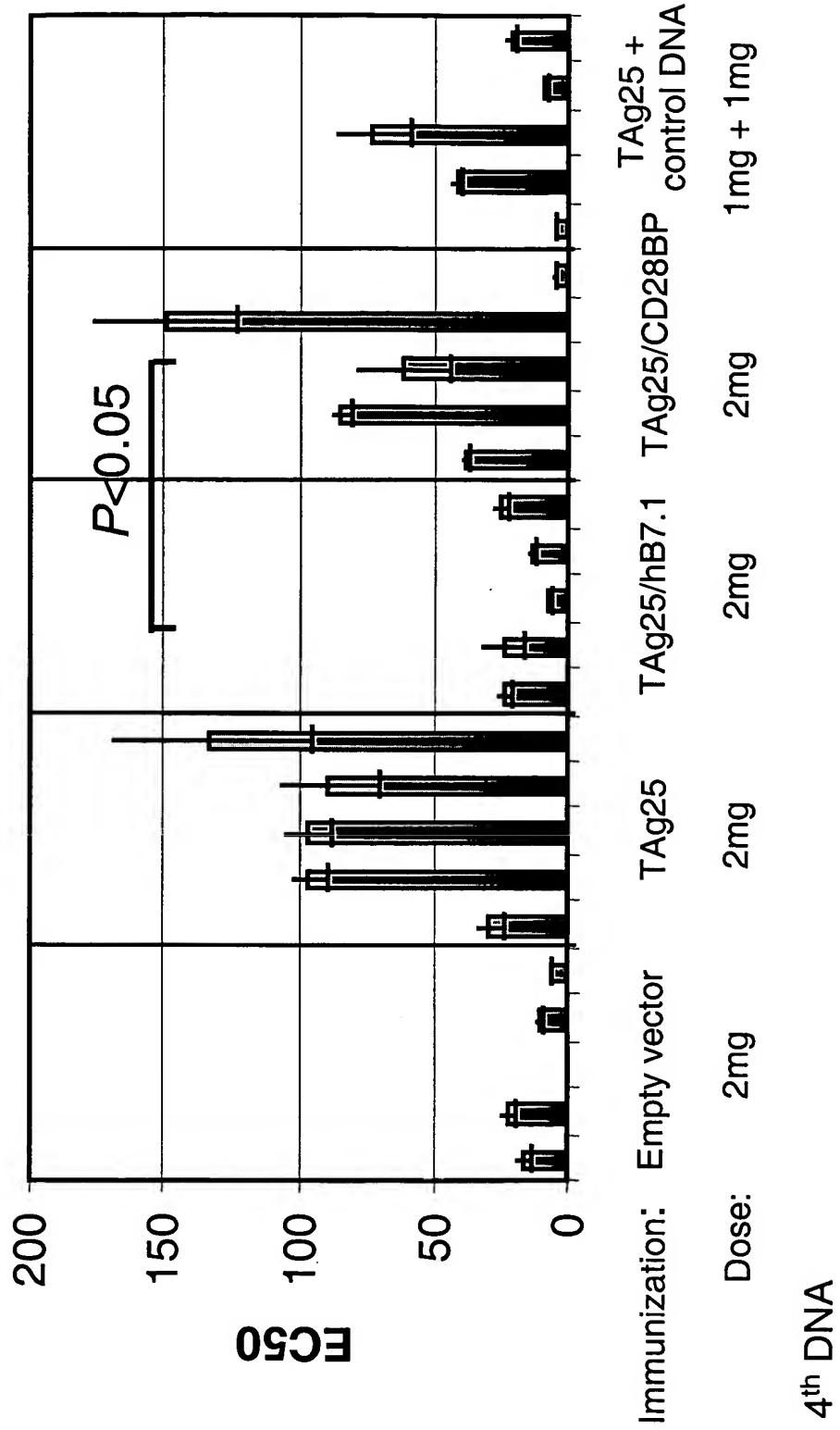
Figure 18

Vaccine	Antigen-specific Response	
	CD4 T Cell*	CD4 & CD8 T Cell*
Empty vector	0/5	0/5
TAg25	1/5	0/5
TAg25/B71	0/5	0/5
<b>TAg25/CD28BP</b>	<b>3/5</b>	<b>2/5</b>

- \* Number of animals positive when restimulated with TAg25
- \*\* Number of animals that are positive for restimulation with both TAg25 and EpCAM peptide mixture in separate cultures

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Figure 19



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Figure 20

